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Molecular docking and dynamic studies of different Histidine derivatives as HDAC2 inhibitors

Geetha Ramakrishnan* and Naresh Kandakatla

Department of Chemistry, Sathyabama University, Jeppiaar Nagar, Chennai, India

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ABSTRACT

Histone deacetylases 2 (HDAC2) proteins belongs to Class I histone deacetylase (HDAC) family and an important target for the treatment of different types of cancer. Of the various HDAC2 inhibitors, our earlier investigations proved that presence of histidine moiety yielded better clinical results. The search of histidine containing compounds is done extensively which yielded a total of 1284 hit compounds. The chosen compounds were subjected to molecular docking in the active site of HDAC2 (PDB: 3MAX) and screening done based on Lipinski rule of 5, resulted in twenty hit compounds as novel potential HDAC2 inhibitors. The careful analysis of the investigation gave the compound ZINC13282319-(2S)-2-(3-aminopropanamido)-3-(3H-imidazol-4-yl)propanoic acid as the most promising compound based on the docking score and hydrogen bond interaction. The best possible interactions of the lead compounds are simulated for stability using molecular dynamics. The results of this investigation provide valuable information on the design of highly selective histidine derivatives.

Key words: HDAC2 inhibitors, Histidine Derivatives, Molecular Docking, Molecular Dynamics simulation

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INTRODUCTION

In eukaryotic cells, nuclear DNA wraps around a protein core consisting of histones H2A, H2B, H3, and H4 to form chromatin, with basic amino acids of the histones interacting with negatively charged phosphate groups of the DNA. Approximately 146 base pairs of DNA wrap around a histone core to make up a nucleosome particle, the repeating structural motif of chromatin. Histones are subject to posttranslational acetylation of the α,ε-amino groups of N-terminal lysine residues. The acetylation reaction is catalyzed by enzymes termed histone acetyl transferase (HATs). Acetylation neutralizes the positive charge of the lysine side chain, and is thought to impact chromatin structure in a manner that facilitates transcription. A family of enzymes termed histone deacetylases (HDACs) has been reported to reverse histone acetylation. Perturbation of this balance is often reversed in human cancers and inhibition of HDACs has emerged as a novel therapeutic strategy against cancer [1].

Histone deacetylases are generally classified into four different classes, namely, HDACs 1–3 and 8, belonging to Class I and related to homologous to Rpd3, HDAC 4–7, 9-10 are Class II related to Hda1, Sirt 1–7 are Class III and are similar to Sir2 and HDAC11 belongs to Class IV. Classes I and II are operated by zinc dependent mechanism and Class III by NAD [2-3]. Histone deacetylases (HDACs) control the gene expression and cellular signalling and histone deacetylases 2 (HDAC2) is overexpressed in solid tumours including colon cancer, lung cancer, cervical carcinoma, breast cancer, and kidney/cervix cancer and also in Alzheimer's disease. Several HDAC inhibitors are in clinical trial, namely, hydroxamic acid derivatives, benzamide derivatives, cyclic peptides, and short-chain fatty acids [4]. The first histone deacetylase (HDAC) inhibitor SAHA (suberoylanilidehydroxamic acid or vorinostat) approved by FDA for treating cutaneous T-cell lymphoma and other hydroxamic acids are in clinical trial. The benzamide derivatives, which are in clinical trials, are Entinostat (MS-275 or pyridin-3-yl methyl 4-((2-aminophenyl) carbamoyl) benzyl carbamate) currently in phase II clinical trial for Hodgkin lymphoma, phase I trial of advanced leukemia and myelodysplastic syndrome (MDS), and Mocetinostat (MGCD0103 or N-(2-

Aminophenyl)-4-[[(4-pyridin-3-ylpyrimidin-2-yl)amino]methyl] benzamide) in phase II clinical trial for Hodgkin lymphoma, phase I trial of advanced leukemia, myelodysplastic syndrome (MDS), diffuse large B-cell lymphoma, and follicular lymphoma [5]. Different histone deacetylase (HDAC) inhibitors had been synthesized and experimental activity was found. Different pharmacophore and virtual screening studies had been reported on histone deacetylase (HDAC) with known hydroxamic acid derivatives and QSAR studies were reported on histone deacetylases 2 (HDAC2) with N(2-aminophenyl)-benzamides [6-7]. In the present study histidine derivatives[8-11]are used for molecular docking studies for histone deacetylases 2 (HDAC2) proteins to find lead compounds for the treatment of different types of cancer.

EXPERIMENTAL SECTION

2.1. Data set preparation:

Histidine containing compounds of nearly 10000 are chosen from Zinc Database and a set of 1284 compounds are extracted based on Lipinski rule of 5. The choice of choosing the histidine moiety in the HDAC inhibitor was based entirely on the previous investigation [12] and certain unpublished results from our laboratory. The chosen compounds were constructed and energy minimized and the final data set comprises of 72 compounds with dock score more than 100.

2.2. Molecular Docking:

Docking is the binding orientation of small molecules to their protein targets in order to predict the affinity and activity of the small molecules. Hence docking plays an important role in the rational drug design. Molecular docking studies were performed by using LigandFit module in Discovery Studio [13]. There are three stages in LigandFit protocol: (i) docking, in which attempt is made to dock a ligand into a user defined binding site, (ii) in situ ligand minimization, and (iii) scoring, in which various scoring functions were calculated for each pose of the ligands. Protein preparation was the main step in docking and all ligands were docked into the active site of the receptor. Protein preparation involves deletion of water molecules and addition of hydrogen atoms and applying CHARMm force field. The active sites were searched using flood filling algorithm. The active site was defined as region of HDAC2 that comes within 10 Å from the geometric centroid of the ligand. Ten poses were generated for each ligand during the docking process and the best poses were selected based on the best orientation of the molecule in the active site and dock score values, which was selected after energy minimization with smart minimization. The dock score was calculated using the following formula:

DOCK SCORE(Force Field) = - {[Ligand/Receptor Interaction Energy]+ Ligand Internal Energy}

Single dock score may fail to obtain active molecules; hence, consensus scoring method was applied which consists of LigScore1, LigScore2, Jain, Piecewise Linear Potential (PLP1 and PLP2), and Potential of Mean Force (PMF). The active molecules were selected based on the consensus scoring method and H-bond interaction with the receptor. The crystal structure of the HDAC2 protein (PDB ID: 3MAX) was downloaded from the protein data bank http://www.rcsb.org/pdb). The crystal structure of histone deacetylases 2 (HDAC2) protein has three chains, which are A, B, and C. The chain A has higher docking score than chains B and C, so chain A is selected for docking. The hit compounds from the database screening with positive Lipinski's drug likeness were subjected to molecular docking studies into the active site of the 3MAX receptor.

2.3. Molecular Dynamics Simulation Study:

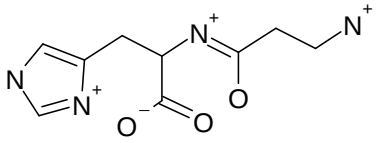
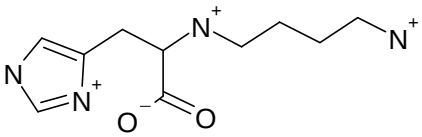
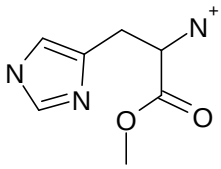
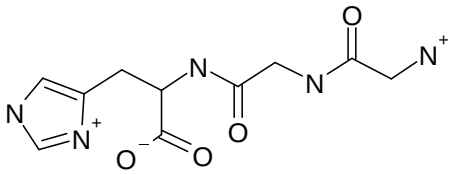
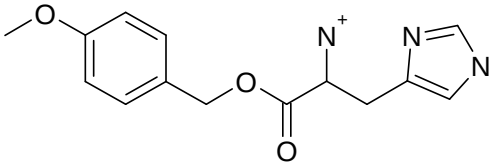
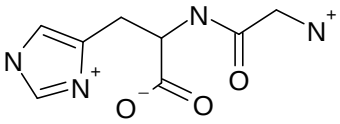
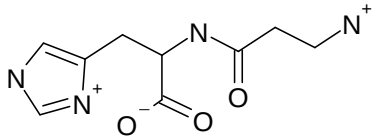
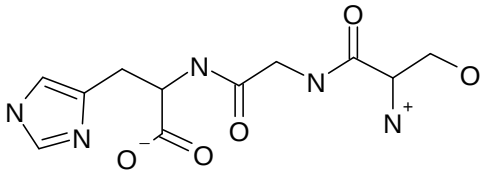
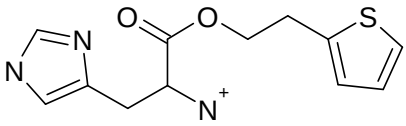
Molecular dynamics studies performed in order to investigate further details of the interaction between the protein and the ligand using simulation package in Discovery studio with CHARMm force filed. Top four selected inhibitor complexes were subjected to a 100 ps NVT (Constant temperature dynamics using Berendsen weak coupling method) molecular dynamic simulation. Implicit solvation by Distance-dependent dielectric was applied to the system in order to simulate in solvent environment. The complexes are energy minimized by the steepest descent and conjugate gradient methods until the system reached 0.001 kcal/mol convergence. System was then subjected to 5 ps heating step from 50 to 300 K, followed by 10 ps equilibrium process to thermally equilibrate the molecules of the systems and finally 100 ps full MD production at 300 K with NVT ensemble. All simulation steps were run with a time step of 1 fs. Full MD trajectory was considered for analysis.

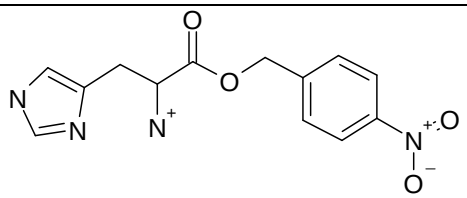
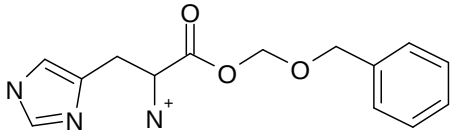
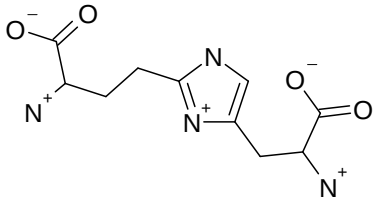
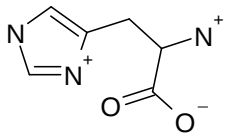
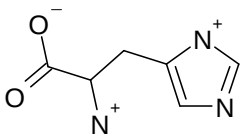
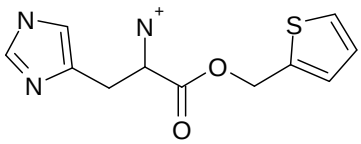
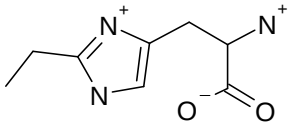
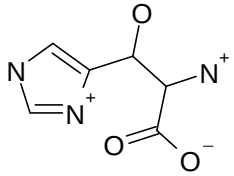
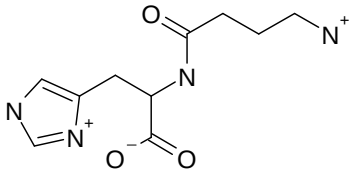
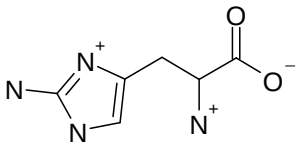
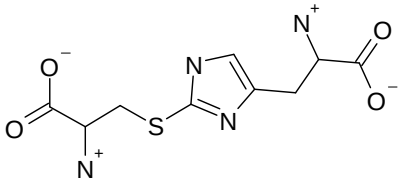
Binding energies were calculated for selected four inhibitors in solvent environment which was constructed for each molecule from average Gibbs energy. The relationship between the Gibbs free energy of ligand, receptor and complex was given in the following equation [14-15].

$\Delta\bar{G}_{binding} = \bar{G}_{complex} - (\bar{G}_{ligand} + \bar{G}_{receptor})$

The average Gibbs energy which was constructed from each energy componentin the above equation is the binding free energy of the complex.

Table 1: The lead compounds with their ID and structure

Compound No	Chemical Name	Zinc ID	Chemical Structure
1	L-Carnosine	ZINC13282319	
2	(2S)-2-(4-aminobutylamino)-3-(1H-imidazol-5-yl)propanoic acid	ZINC78283465	
3	H-His-OMe. 2HCl	ZINC04634894	
4	H-Gly-Gly-His-OH-H_2O	ZINC17127936	
5	(4-methoxyphenyl) methyl Histidine	ZINC40954038	
6	Gly-His	ZINC35024721	
7	beta-Alanyl-L-histidine	ZINC13282321	
8	(2S)-2-[[2-[[(2R)-2-amino-3-hydroxy-propanoyl] amino]acetyl]amino]-3-(1H-imidazol-4-yl)propanoic acid	ZINC39816859	
9	2-(2-thienyl) ethyl histidine	ZINC49585026	

10	(4-nitrophenyl) methyl histidine	ZINC60071287	
11	benzyloxy methyl histidine	ZINC79164507	
12	2-(3-Carboxy-3-aminopropyl)-L-histidine	ZINC40165408	
13	alpha-methyl histidine	ZINC4899498	
14	H-DL-His-OH.HCl.H2O	ZINC19014871	
15	2-thienyl methyl histidine	ZINC49584674	
16	(2R)-2-amino-3-(2-ethyl-1H-imidazol-4-yl)propanoic acid	ZINC29404495	
17	3-hydroxyhistidine	ZINC13283580	
18	homocarnosine	ZINC38606076	
19	(2S)-2-amino-3-(2-amino-1H-imidazol-4-yl)propanoic acid	ZINC26286704	
20	2-ammonio-3-((4-(2-ammonio-2-carboxylatoethyl)-1H-imidazol-2-yl)thio)propanoate	ZINC27735780	

RESULTS AND DISCUSSION

The protein HDAC2 was downloaded from PDB and it has 3 chains namely A, B and C, Chain A was selected for docking studies [12] and the protein chain A was docked with the prepared histidine derivative ligand set of 1284 from ZINC database. Of the 1284 compounds docked with the HDAC2 receptor there were 72 compounds showed interaction by binding at the HDAC2 binding site residues with a dock score of above 100. The docking score and hydrogen bond interactions were consider for selecting the best pose of the docked compounds. MS-275 (Entinostat) is chosen as reference compound for comparing the dock score of compounds. The results of docking score and H-bonds of top 20 compounds were listed in Table 1 and 2; chemical structure of all listed 20 compounds given supplementary table 1. and all compounds were ranked by dock score. MS-275 (Entinostat) has the dock score of 42.6 and 4 H-bond interaction with ARG39, CYS156, GLY305, HIS183 (Ref.ADFB). The compounds which shows higher Dock score and H-bonds with crucial amino acids were consider as effective lead compounds for HDAC2 inhibition. Chemical structure top selected four histidine compounds (ZINC13282319, ZINC78283465, ZINC49585026 and ZINC40165408) were shown in **figure 1** and docking pose of each ligand displayed in **figure 2.** ZINC13282319 ((2S)-2-(3-aminopropanamido)-3-(3H-imidazol-4-yl)propanoic acid or L-Carnosine) has the dock score of 159. 55, 10 H-bonds with HIS145, CYS156 (3), GLY142, ASP181 (3), ASP269 (2) amino acids. For ZINC78283465 (2-((4-ammoniobutyl) ammonio)-3-(1H-imidazol-3-ium-4-yl)propanoate) the Dock score is 145.86, the docked ligand has 8 H-bonds with TYR29, GLY142, CYS156, ASP269 (2), ASP181 (3) amino acids. ZINC49585026 (3-(1H-imidazol-4-yl)-1-oxo-1-(2-(thiophen-2-yl)ethoxy)propan-2-aminium) has the Dock score of 126.72 and 7 H-bonds with TYR308, HIS145 (2), ASP181 (4) amino acids. ZINC40165408 (2-ammonio-4-(4-(2-ammonio-2-carboxylatoethyl)-1H-imidazol-3-ium-2-yl)butanoate) has the Dock score of 122.90, having 7 H-bonds with ARG39, GLY143, ALA141, HIS145, ASP181 (3).

Table 1: Identified top 20 compounds with Dockscore

ZINC Compound No	LigScore1	LigScore2	PLP1	PLP2	Jain	PMF	DockScore	Lig Interaction Energy
ZINC13282319	4.65	3.26	75.72	71	9.21	60.3	159.555	7.99
ZINC78283465	4.42	2.96	64.76	65.64	8.2	60.71	145.862	14.282
ZINC04634894	3.7	4.41	68.26	44.8	4.29	83.22	144.381	0.291
ZINC17127936	3.42	3.02	72.74	63.5	9.1	68.18	138.573	1.5
ZINC40954038	3.78	4.14	102.24	79.53	8.54	139.23	131.978	1.894
ZINC35024721	4.54	3.88	76.71	61.11	7.66	61.79	131.068	0.467
ZINC13282321	4.23	3.38	69.2	56.24	8.94	64.43	130.923	22.253
ZINC39816859	4.96	3.45	76.18	71.85	9.94	88.5	127.525	3.143
ZINC49585026	4.49	5.13	88.09	75.46	5.31	126.95	126.728	1.903
ZINC60071287	4.4	4.37	100.15	78.5	5.78	122.92	126.065	0.954
ZINC79164507	4.05	3.94	74.84	69.55	10.97	110.85	125.692	0.273
ZINC40165408	5.36	4.4	111.43	83.47	13	72.57	122.901	1.316
ZINC4899498	4.09	4.38	68.68	45.24	6.68	79.49	122.605	-0.337
ZINC19014871	3.37	3.66	50.06	33.49	4.67	89.64	122.428	4.113
ZINC49584674	3.47	3.19	56.86	55	6.55	83.83	122.385	-3.151
ZINC29404495	3.86	3.98	75.46	55.51	7.39	85.06	121.687	-0.92
ZINC13283580	4.52	4.18	67.49	53.24	7.43	95.31	121.625	0.521
ZINC38606076	4.55	3.55	62.3	52.58	9	68.59	121.032	4.748
ZINC26286704	4.46	4.51	83.1	55.09	5.95	74.32	120.976	-0.6
ZINC27735780	4.78	4.53	87.84	61.94	7.31	93.28	120.71	-0.599

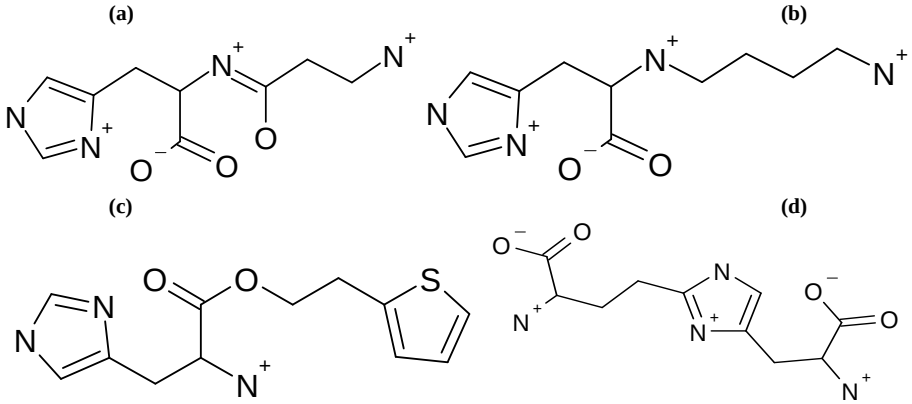


Figure 1: Chemical Structures of selected four compounds (a) ZINC13282319 (b) ZINC78283465 (c) ZINC49585026 (d) ZINC40165408

Table 2: Identified top 20 compounds with H-bond interaction

ZINC Compound No	H-bond Interaction	H-bond distance
ZINC13282319	HIS145, CYS156 (3), GLY142, ASP181 (3), ASP269 (2)	2.44, 2.43, 2.41, 1.88, 1.92, 1.66, 1.80, 2.28, 2.47, 1.64
ZINC78283465	TYR29, GLY142, CYS156, ASP269 (2), ASP181 (3)	1.93, 2.35, 2.14, 1.66, 2.41, 1.89, 1.67, 1.99
ZINC04634894	ASP181 (2), HIS145	1.91, 1.79, 1.72
ZINC17127936	ASP269, ASP181 (3)	1.70, 2.49, 1.63, 1.72
ZINC40954038	TYR308, ASP181 (4), ASP269	2.38, 1.92, 1.90, 2.39, 2.03, 1.73
ZINC35024721	GLY306, GLY142, ASP181 (3), ASP269	2.15, 2.07, 1.64, 1.67, 2.45, 1.77
ZINC13282321	CYS156, GLY142, ASP269, ASP181 (2)	2.23, 2.34, 1.68, 1.75, 1.76
ZINC39816859	ARG39, TYR308, ASP181 (3), ASP269	1.92, 2.01, 2.36, 1.65, 1.77, 1.86
ZINC49585026	TYR308, HIS145 (2), ASP181 (4)	2.48, 2.41, 2.38, 1.79, 2.39, 1.81, 1.90, 2.48
ZINC60071287	ARG39, HIS145 (2), ASP181 (3)	2.00, 2.36, 1.85, 2.31, 1.87, 1.71,
ZINC79164507	TYR308, ASP181 (4), ASP269	2.01, 1.86, 2.30, 2.15, 1.76, 1.68
ZINC40165408	ARG39, GLY143, ALA141, HIS145, ASP181 (3)	1.72, 2.07, 1.60, 1.78, 2.45, 1.78, 2.15
ZINC4899498	HIS183, GLY154, HIS145, ASP181 (2)	2.44, 2.10, 1.73, 1.88, 1.75
ZINC19014871	ASP269 (2), ASP181 (3)	1.80, 2.19, 2.41, 1.63, 1.74
ZINC49584674	TYR308, ASP181 (3), ASP269 (2)	1.79, 1.76, 2.23, 1.84, 2.42, 2.27
ZINC29404495	GLY143, ASP269, ASP181 (3)	2.27, 1.90, 1.68, 1.89, 2.11
ZINC13283580	GLY154, ASP269 (2), ASP181 (3), HIS145	2.07, 1.83, 1.94, 2.47, 1.66, 2.05, 2.06
ZINC38606076	TYR29, ALA141, ASP181 (3), ASP269	2.46, 2.05, 1.61, 1.77, 2.37, 1.69
ZINC26286704	GLY143, ASP181 (2), HIS145, GLY142	1.91, 1.90, 1.78, 1.73, 2.14
ZINC27735780	ARG39 (4), ALA141 (2), ASP181 (2), HIS145	2.25, 1.83, 2.30, 2.40, 2.16, 1.62, 2.01, 1.71, 1.71

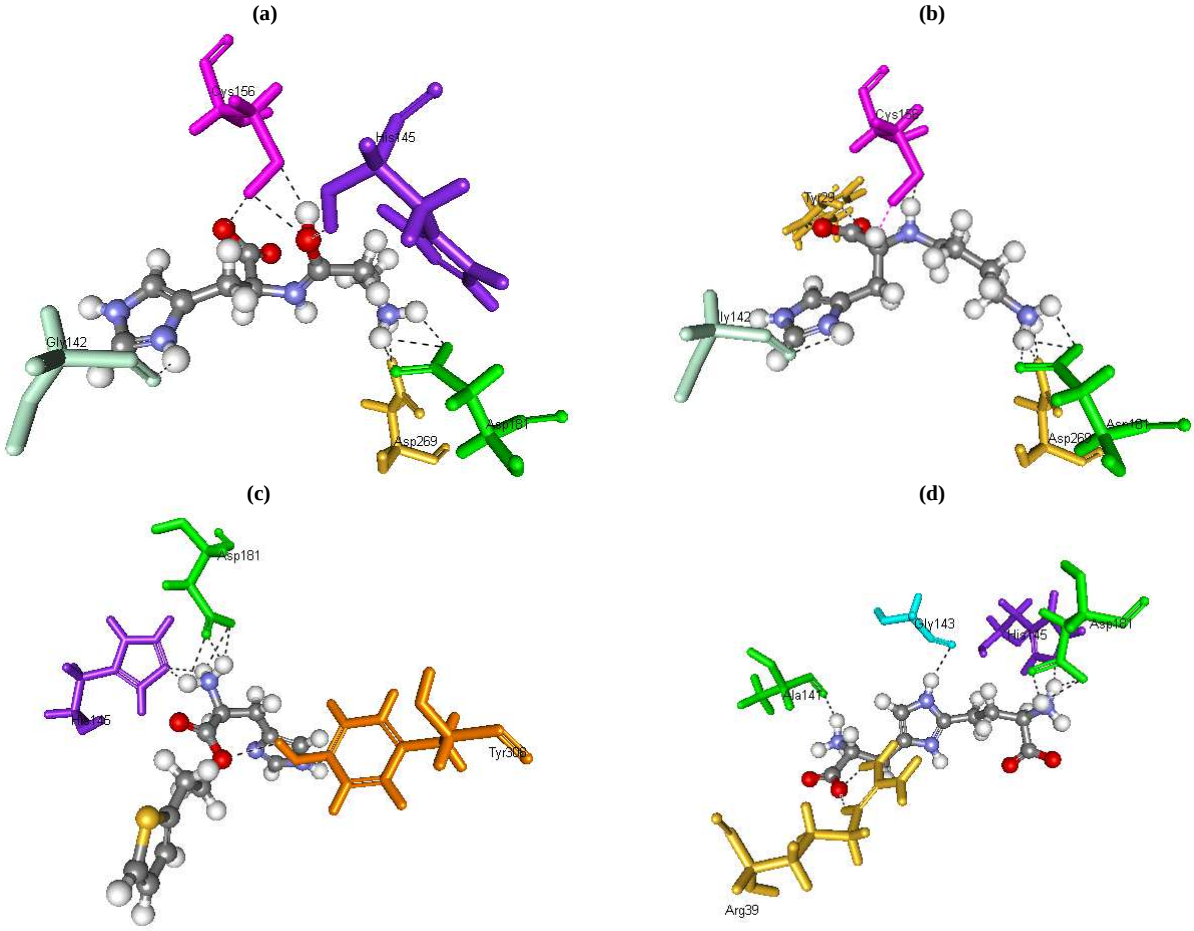


Figure 2: Docked poses selected four compounds (a) ZINC13282319 (b) ZINC78283465 (c) ZINC49585026 (d) ZINC40165408

Correlation between dock score and molecular weight of top selected 20 compounds shows compounds which have molecular weight above 250 has average dock score of 126.87, whereas compounds which are below the molecular weight of 250 has average dock score of 130.8 (figure 3a) and the correlation between molecular weight and H-bonds shows that compounds above 250 molecular weight has average 6.3 H-bonds and for below 250 molecular weight has 5.9 H-bonds (figure 3b). In conclusion compounds above the molecular weight of 250 shows good dock score along with better H-bond interaction.

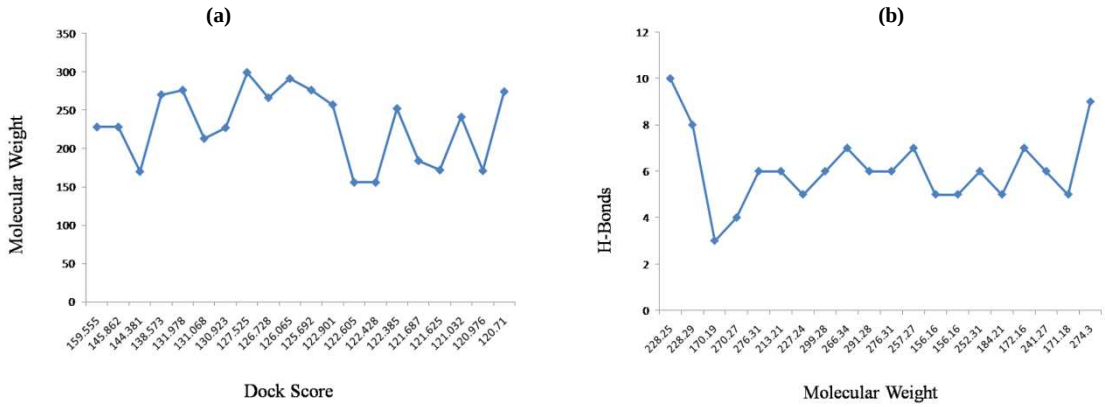


Figure 3: (a) Correlation graph between Dockscore vs Molecular weight; (b) Correlation graph between Molecular weight vs H-bonds

The best poses of selected protein-ligand complexes HDAC2- namely ZINC13282319, ZINC78283465, ZINC49585026 and ZINC40165408 were subjected to molecular dynamic studies.

Molecular dynamics simulation has been done to check the stability and interaction of structure during the simulation. The binding free energy is able to determine the ability of enzyme protein to bind its substrate. In this study, binding free energies were calculated in solvent environment. The binding energy of the each complex was listed in Table 3. The results showed that HDAC2 - ZINC49585026 has lower binding energy than other complexes, which indicates that higher stability and greater binding affinity in the active site of the receptor.

Table 3: Binding Energies of selected four inhibitors

Binding Free energies in solvent (kcal/mol)			
ZINC13282319	ZINC78283465	ZINC49585026	ZINC40165408
-112.46	-115.88	-99.92	-125.07

The stability of four inhibitors (ZINC13282319, ZINC78283465, ZINC49585026 and ZINC40165408) has been monitored by plotting the Root mean square deviation (RMSD) of the complex with respect to simulation time scale (figure 4). Where it shows that during simulation the RMSD fluctuated with increased value. The RMSD for the structure in the starting state was at 0.9 Å for all the compounds and ended at 1.5 Å for the compounds represented by ZINC13282319, ZINC78283465and ended at 1.6 Å for the compound represented by ZINC49585026and ended at 1.4 Å for the compound represented by ZINC40165408during 60 ps, the RMSD began to increase until the distance1.96 Å for ZINC49585026), 1.8 Å for ZINC782834650, 1.6 Å for ZINC13282319and 1.4 Å for ZINC40165408mark around 100 ps. The RMSD of the four inhibitors are compared and the compound represented by ZINC49585026 in the active site is relatively high (showed as blue line). The fluctuation of RMSD up to 2 Å was indicated and the order was found to be ZINC78283465, ZINC13282319 and ZINC40165408. The RMSD graph suggests that the complex tend to be stable and flexible throughout the 100ps MD simulation.

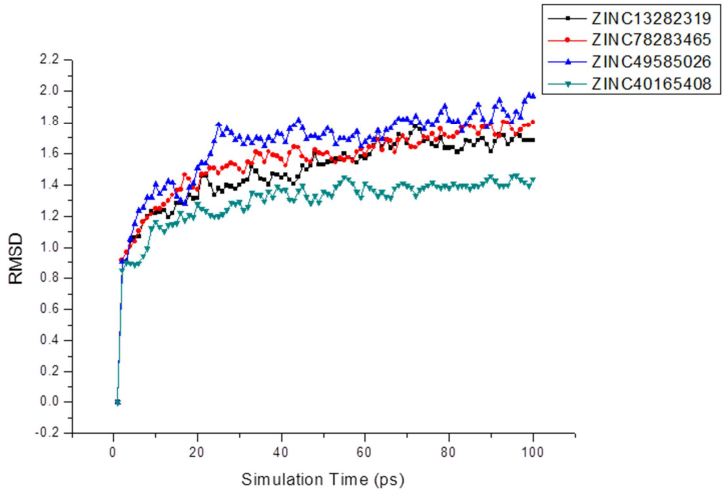


Figure 4: RMSD plots of the four HDAC2 – inhibitor complexes

Potential energy is a measure to indicate the stability of the system. Hence the potential energy of the four inhibitors complexes were monitored by plotting them against simulation time (figure 5a). The potential energy remained stable throughout the simulation and the computed energy value of four inhibitors ZINC13282319, ZINC78283465, ZINC49585026, and ZINC40165408 found to be -5140.93 kcal/mol, -5,088.34 kcal/mol, -5,240.19 kcal/mol and -5,320.90 kcal/mol respectively. Total energy of four inhibitors (ZINC13282319, ZINC78283465, ZINC49585026, and ZINC40165408) with respect to time was found to decrease on a constant basis(Figure 5b).

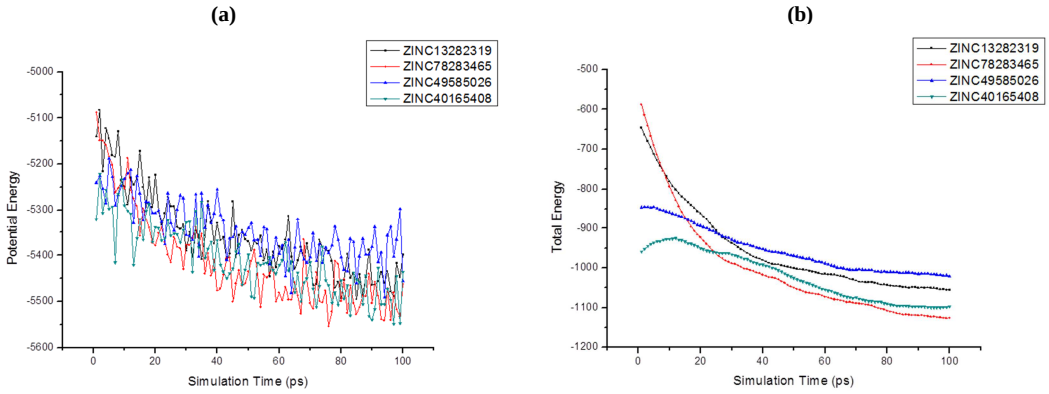


Figure 5: (a) Potential energy vs simulation time (ps) (b) Total energy vs simulation time (ps) plots of four inhibitor complexes

The hydrogen bond is able to indicate the strength of the interaction. The better the interaction resulted in more number of hydrogen bonds between them. The docked complexes show significant hydrogen bonding patterns. The intermolecular hydrogen bonding between protein ligand complexes plays important role in stability of the system. The total number of intermolecular hydrogen bonds formed between the complexes was showed in figure 6. The number of hydrogen bonds formed in the HDAC2 – inhibitor interaction site observed during the simulation and found that in all inhibitor complexes, the number of H-bonds formed had increased. In HDAC2-ZINC13282319 complex the number of H-bonds increased from 10 to 18, where as in HDAC2- ZINC78283465 complex is from 8 to 17. The HDAC2- ZINC49585026 complex shows increased H-bonds from 7 to 10 and in HDAC2-ZINC40165408 complex from 7 to 17. Overall results studies shows increased H-bonds during the simulation suggests that all inhibitor complexes shows better interaction in the active site.

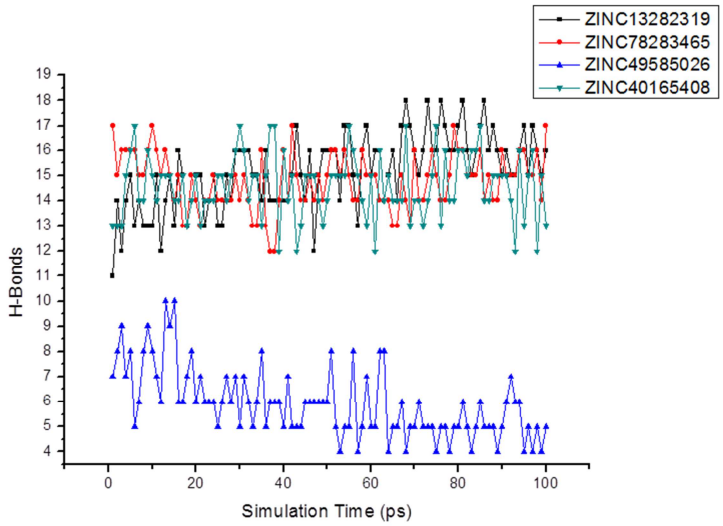


Figure 6: Number of H-bonds vs simulation time (ps) of four inhibitor complexes

CONCLUSION

The histidine moiety is found to play an important role in the design of HDAC2 inhibition. Based on the current investigation, ZINC13282319 (L-Carnosine), ZINC78283465((2S)-2-(4-aminobutylamino)-3-(1H-imidazol-5-yl) propanoic acid), ZINC49585026(2-(2-thienyl) ethyl histidine), ZINC40165408 (2-(3-Carboxy-3-aminopropyl)-L-histidine) are selected as lead compounds for further fruitful experimental work to prove the drug efficacy. The molecular dynamics done on these compounds show greater stability in the order ZINC49585026, ZINC78283465, ZINC13282319 and ZINC40165408.

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